Nig. J. Physiol. Sci. 25(December 2010) 129 – 134
www.njps.physocnigeria.org

Nig. J. Physiol. Sci.

Effects of Methionine Containing Paracetamol Formulation on Serum Vitamins and Trace Elements in Male Rats.

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Summary: Methionine is an effective antidote in the treatment of paracetamol-induced toxicity but at large doses it has been reported to induce or aggravate a number of pathological conditions. It also alters plasma levels of many vital elements and molecules. This study was designed to identify if the alteration observed for antioxidant vitamins and minerals especially at sub-toxic and toxic levels of exposure in our earlier study of 24-hour exposure period may warrant trace elements supplementation. This was investigated by carrying out a 48-hour study to test the ability of a living organism to restore homeostasis of these vital molecules and elements. The levels of antioxidant minerals and vitamins were estimated in the serum samples obtained from adult male Wistar rats exposed to paracetamol tablets. At 100 mg\kg BW (body weight) vitamin A, niacin, riboflavin, selenium and manganese were not significantly different from the control group ($p>0.05$). Moreover at 350 mg\kg, all these indices except zinc were not significantly different in the exposed group compared with controls ($p>0.05$) whereas at 1000 mg\kg level of exposure manganese, selenium and vitamin E were not significantly decreased at the end of 48 hours of exposure but copper, niacin and vitamin A were significantly increased in the exposed group compared with the controls ($p<0.05$). These results suggest that with time the body may be capable of bringing about restoration of the levels of some of these elements\vitamins. This was more evident at 350 mg\kg level of exposure than a higher dose of 1000 mg\kg level.
Keywords: Paracetamol formulation, Vitamins, Trace elements

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Manuscript received: November, 2010

INTRODUCTION

Acetaminophen, a standard analgesic and antipyretic agent for the treatment of pyrexia and different pain states (Insel, 1996) has been reported to be hepatotoxic and nephrotoxic at overdose level (Cohen &Khairallah, 1997; Lotivitz, 1995). At toxic level it yields the reactive species; N-acetyl- para-benzoquinoneimine (Borne, 1995) which attacks cellular components (e.g. proteins) resulting in centrilobular necrosis (Hemabarrathy *et al*., 2009). Glutathione, an important antioxidant which binds this reactive species (Kozer *et al*., 2003) requires the enzyme glutathione peroxidase; selenium is an important cofactor for this enzyme (Kozer *et al*., 2003; Hill & Burk, 1994). The role of many antioxidants in acetaminophen-induced toxicity has been investigated through a number of studies (Kozer *et al*., 2003; Kröger *et al*., 1996) and there are reports to indicate that many of them indeed affect the metabolism of paracetamol and modulate its toxicity.

Kröger *et al*. (1996) reported that niacin in form of nicotinic acid amide inhibited acetaminophen-induced injury in mice and therefore suggested its use in combination form with acetaminophen to avoid hepatic injury in human subjects who have higher sensitivity to its exposure or to minimize hepatic injury as a result of overdose. Jamshidzadeh *et al*. (2008) have also reported the hepatoprotective effects of lycopene through its antioxidant property in acetaminophen exposed rats.

This modulatory role played by these antioxidants and the implication of altered levels of these elements and biomolecules as a result of exposure to high doses of methionine (Ferret *et al*., 2001) may warrant a study of this nature, especially

as there is evidence to show that methionine has hepatoprotective effect (Neuvonen *et al*., 1985; Krenzelok, 1997) and is being included in acetaminophen in some parts of the world and offered for sale by many pharmaceutical companies. This may have consequential alteration in the plasma levels of antioxidants such as zinc, selenium and vitamin E.

Moreover, the result of another study of shorter exposure period (24 hours) showed significant alterations in the concentrations of niacin, riboflavin, vitamins A and E and the antioxidant minerals compared with controls at one level of exposure or the other. The aim of this study is to determine the serum levels of riboflavin, niacin, vitamins A & E, Zn, Cu, Mn, and selenium after 48 hours of exposure to acetaminophen formulation so as to observe if without any form of intervention the body's homeostatic mechanisms in male Wistar rats can restore the depleted serum levels of these vitamins and minerals which had been reported to have occurred in our earlier study (Iyanda *et al*., 2011) of shorter duration of exposure (24 hours).

MATERIALS AND METHODS

Animals and drug regimen: All the experimental animals were handled in compliance with internationally accepted principles for laboratory animals' use and care as found in US guidelines (NIH publication\85-23, revised in 1985). Twenty four male Wistar rats (200-240g) were obtained from the animal house of the Department of Veterinary Physiology, University of Ibadan. The animals were kept in cages and left to acclimatize for two weeks before the commencement of the experiment. During this period they were given food and water ad libitum. The animals were randomly divided into four groups consisting of six rats per group and each group received one of the following doses of the paracetamol formulation – 0 mg\kg BW (control, received only physiologic saline), 100 mg\kg BW, 350 mg\kg BW & 1000 mg\kg BW, these doses were employed in accordance with the report of Trumper *et al*. (1992). The paracetamol formulation which contained paracetamol- methionine in the ratio of 9:1 was dissolved in pathogen-free physiologic saline prior to administration and the drug was administered through intra-peritoneal route. Mc Lean & Day (1975) had noted the hepatoprotective ability of paracetamol:methionine combination (ratio 9:1) in rats.

Sample preparation and biochemical analysis: Forty-eight hours after drug administration, the animals were sacrificed. Blood samples were collected from the rats through cardiac puncture, and were left to clot and centrifuged at 3000 r.p.m. to obtain serum. The sera were stored at -20°C until the time of analysis. Flame atomic absorption spectrometry was used for the estimation of the antioxidant minerals; zinc, copper, selenium and manganese, Buck Scientific® 205 Atomic Absorption Spectrophotometer was used for this purpose (east Norwalk, USA). High performance liquid chromatography (HPLC) was used for the estimation of vitamin A, vitamin E, niacin and riboflavin.

Statistical analysis: Student 't' test was employed to test the level of significance between the control and each exposure level on one hand and level of significance between one exposure level and another, SPSS version 15 was used for this purpose. Pearson's correlation coefficient was employed to ascertain association between parameters. Values of $P<0.05$ was considered as significant**.**

RESULTS:

Biochemical result

The levels of antioxidant vitamins are presented in Table 1. The serum concentrations of niacin and riboflavin in acetaminophen\methionine exposed male Wistar rats are not significantly different in comparison to the control at 100 mg\kg and 350 mg\kg levels of exposure($p>0.05$), but niacin is significantly increased and riboflavin significantly decreased at 1000 mg\kg in comparison with control ($p<0.05$). Vitamin E at 350 & 1000 mg\kg as well as vitamin A at 100 & 350 mg\kg levels of exposure are not significantly ($p>0.05$) different compared with controls while both vitamin E and vitamin A were significantly increased at 100 mg\kg and 1000 mg\kg respectively ($p<0.05$).

Table 1 also shows inter-exposure group comparison between 100 mg\kg & 350 mg\kg; 100 mg\kg & 1000 mg\kg; 1000 mg\kg & 350 mg\kg. Niacin was significantly different at all levels of comparison. On the other hand, riboflavin and vitamin A are not significantly different at all levels of comparison except for 100 mg\kg & 350 mg\kg where significant changes were observed ($p<0.05$) whereas vitamin E was significantly different at 100 mg\kg & 350 mg\kg as well as 100 mg\kg & 1000 mg\kg comparison levels.

The levels of antioxidant minerals are presented in Table 2. The serum concentrations of both manganese and selenium are not significantly different at all levels of exposure compared to control ($p>0.05$). Zinc and copper, on the other hand, were significantly different at all levels of exposure

($p<0.05$) except that copper was not significantly different at 350 mg\kg level compared with control ($p>0.05$).

Table 3 shows the result of intra-group correlation among antioxidant indices (vitamins and minerals) in acetaminophen exposed male Wistar rats. Manganese, zinc and selenium show no correlation with other indices at all levels of exposure ($p>0.05$). Moreover, there is no correlation between copper and vitamin A & E; as well as between vitamin E and vitamin & riboflavin ($p>0.05$) at all levels of exposure. But correlation was recorded for niacin and riboflavin ($r=0.924$; $p=0.025$)and niacin and vitamin E ($r=0.901$; $p=0.037$) only at 100mg\kg level. In addition correlation was recorded between niacin and copper ($r=0.900$; $p=0.019$) also only at 350 mg\kg. Vitamin A shows correlation with niacin ($r=0.901$; $p=0.037$) and riboflavin ($r= -0.907$; $p=0.34$).

The results of this study are shown in the tables below**.**

Table 1

Antioxidant Vitamins in Acetaminophen Exposed And Control Male Wistar Rats — 48 Hour Study

EXPO. GROUPS	NIACIN *ng\ml*	RIBO. *µg\dl*	VIT.E *mg\L*	VIT.A *µg\dl*
1.	1.64±0.26	48.84±1.75	4.83±0.30	28.30±2.38
2.	1.24±0.31	48.45±1.78	7.78±0.94	29.22±1.20
3.	1.78±0.33	47.3±1.77	4.73±0.33	30.85±1.40
4.	2.42±0.28	37.62±2.55	5.00±0.44	46.14±4.83
P value comparison table.				
1-2	NS	NS	0.000*****	NS
1-3	NS	NS	NS	NS
1-4	0.002*****	0.000*****	NS	0.000*****
2-3	0.029*****	NS	0.000*****	NS
2-4	0.000*****	0.000*****	0.000*****	0.001*****
3-4	0.003*****	0.000*****	NS	0.002*****

Abbreviations: RIBO., riboflavin; VIT.A, vitamin A; VIT.E, vitamin E.
Results are expressed as mean ±standard deviation; $p< 0.05$ is considered significant. NS- not significant.
Group 1- control; Group 2- 100mg\kg body weight; Group 3- 350mg\kg body weight; Group 4- 1000mg\kg body weight

TABLE 2

Antioxidant Minerals In Acetaminophen Exposed and Control Male Wistar Rats — 48 Hour Study.

EXPO. GROUPS	Zinc *µg\dl*	Copper *µg\dl*	Mn*µg\dl*	Se *µg\dl*
1.	74.96±2.66	87.1±5.81	3.75±1.25	43.2±24.88
2.	89.14±1.88	76.4±3.87	2.63±0.42	42.9±42.76
3.	67.02±3.47	84.04±3.99	2.91±26.20	41.06±26.16
4.	70.26±1.00	116.1 ±10	3.46±1.48	39.80±14.11
P value comparison table.				
1-2	0.000*****	0.009*****	NS	NS
1-3	0.004*****	NS	NS	NS
1-4	0.006*****	0.000*****	NS	NS
2-3	0.000*****	0.015*****	NS	NS
2-4	0.000*****	0.000*****	NS	NS
3-4	NS	0.001*****	NS	NS

Abbreviations: Mn, manganese; Se, selenium.

Results are expressed as mean ±standard deviation; $p< 0.05$ is considered significant. NS- not significant.

Group 1- control; Group 2- 100mg\kg body weight; Group 3- 350mg\kg body weight; Group 4- 1000mg\kg body weight

TABLE 3
Intra-Group Correlation between Indices in Acetaminophen Exposed Male Wistar Rats.

	Niacin	ribo.	Vit. A	Vit. E	Zinc	Copper	Mn	Se
Niacin	-	†	¥	§	NS	*	NS	NS
Ribo.		-	Δ	NS	NS	‡	NS	NS
Vit. A			-	NS	NS	NS	NS	NS
Vit. E				-	NS	NS	NS	NS
Zinc					-	NS	NS	NS
Copper						-	NS	NS
Mn							-	NS
Se								-

Abbreviations & Keys: ribo., riboflavin; Vit. A, vitamin A; Vit. E, vitamin E; Mn, manganese; Se.,selenium.
†-significance recorded only at 100mg\kg BW (body weight) level of exposure (r=0.924; p=0.025).
§- significance recorded only at 100mg\kg BW level of exposure (r=0.901; p=0.037)
*- significance recorded only at 350mg\kg BW level of exposure (r=0.900; p=0.019).
¥- significance recorded only at 1000mg\kg BW level of exposure (r=0.901; p=0.037).
Δ- significance recorded only at 1000mg\kg BW level of exposure (r= -0.907; p=0.34).
‡- significance recorded only at 0mg\kg BW level of exposure (controls) (0.891; r=0.043).
NS- No significance recorded only at any level of exposure.
P< 0.05 is considered significant.

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DISCUSSION

Zinc, copper, manganese and selenium are inorganic compounds essential for life found in tissues in milligram per kilogram level or less (Nielsen, 1980). They have also been described as substances that make up less than 0.01 percent of the dry weight of the body (Mertz, 1981; Nielsen, 1990). These micronutrients are known to specifically play roles in many physiological process; for example copper, manganese and zinc are an integral component of many metalloenzymes e.g. superoxide dismutase (Ozcelik&Uzun, 2008). Moreover, being an integral part, copper is bound to other proteins and released to play important catalytic roles in detoxification of reactive species (Shenkin *et al*., 2006). Smith *et al*. (2008) have also established that copper deficiency may increase susceptibility to oxidative damage.

Zinc and copper have been reported to interact with one another in their antioxidant roles. Graetke& Chow (2003) have identified a number of biomolecules capable of modulating the cellular effects of copper. Vitamin E protects against copper induced oxidative damage in in vitro and cell culture studies, Wilson's and Menkes diseases – disorders due to disruption of copper homeostasis have been linked to increase in oxidative damage(Power and Jackson, 2008).

The result of our earlier study (Iyanda *et al*., 2011) revealed a significant alteration in the levels of niacin, vitamins A & E, zinc, copper, manganese and selenium in strain, sex and aged-matched rats after 24 hours of exposure at 100 mg\kg BW; 350 mg\kg BW as well as 1000 mg\kg BW levels of exposure prompted the need to identify if the body is able to restore homeostasis after depletion. Moreover, Trumper *et al*., 1992, using indicators of hepatic damage as indices of study reported that the peak of paracetamol toxicity is around the 24th hour. Therefore, this study suggests the possibility of cessation in free radical generation and a decrease demand for antioxidant minerals after which the levels of many of these essential elements and vitamins are being restored through a number of physiologic mechanisms.

One of such may be homeostasis; especially as adjustment in rates of intestinal absorption, excretion and release from intracellular store has been reported to be a common feature of many of these elements, especially in instances of acute depletion (Shenkin *et al*., 2006). The non-significant difference in the serum levels of vitamin E, selenium and manganese in treated rats compared with controls at 1000 mg\kg BW exposure level may suggest an interplay of some of the homeostatic mechanisms, since no pretreatment, intervention or supplementation of any of these vitamins and elements was carried out.

Adjustment in intestinal absorption is possible because studies have revealed that only 30 – 50 percent of these micronutrients are absorbed by a mammal on a normal diet (Shenkin *et al*., 2006). Moreover, active transport mechanisms involving absorption by zinc and copper-binding proteins have been reported to upregulate or down-regulate rate of absorption to suit body need. Furthermore a number

of storage proteins e.g. metallothionein play a role in the regulation of these elements (Nielsen *et al*., 1980).

The results of non-significant difference in the levels of niacin, riboflavin, vitamin A, vitamin E, copper, manganese, selenium by the end of the 48th hour in rats exposed to sub-toxic dose of 350 mg\kg BW are an indication that such possible interplay is not limited to the toxic dose. This as well as slight increases in the serum levels of niacin, vitamin A and copper observed for the 3000 mg/kg may rule out the need for trace elements and vitamins supplementation, since many of them are not only antioxidants but may induce oxidative stress at above physiologic levels. For instance copper at toxic level generates reactive hydroxyl radicals, and data from (in vitro and cell culture) have supported the role of copper in initiating oxidative damage and therefore interrupt many important cellullar processes at levels above the physiologic range (Gaetke& Chow, 2003).

The non-significant difference in selenium and manganese levels is in agreement with the observation of Lei *et al*. (2007), Zhu and Lei (2006) & Ishida *et al*. (2004), these workers noted that with all the evidence for the involvement of essential trace elements in acetaminophen toxicity that double null of selenium-glutathione peroxidase-1 and copper, zinc-superoxide dismutase enhanced resistance of mouse primary hepatocytes to acetaminophen toxicity. This is not surprising as the results of this study also showed that selenium was not significantly different at 100 mg\kg; 350 mg\kg and 1000 mg\kg BW levels of exposure compared to controls, this signifies that it played no significant role at all levels of exposure. This was more evident in the fact that it was the only element that did not show any statistical difference when all exposure groups were compared.

In conclusion, the results of this study suggested, the possibility of the body restoring homeostatic serum levels of essential elements and vitamins depleted as a result of paracetamol dosing in Wistar rats. Therefore vitamin and mineral supplementations may not be required in such cases except for zinc which was still decreased at all levels of exposure.

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